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Polyalkyl cyanoacrylate nanocapsules

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In-situ polymerized methyl cyanoacrylate (MCA), ethyl cyanoacrylate (ECA), and butyl cyanoacrylate (BCA) were used to prepare nanocapsules of fluorescein or doxorubicin as markers by a w/o emulsion interfacial polymerization technique. Different concentrations of MCA were also used to show the effect of monomer concentration. The nanocapsules were characterized by electron microscopy, particle size analysis, holding capacity and in-vitro release of the marker substances. After selection of the polymerization solvent system, nearly spherical nanocapsules were obtained using each of the monomers. Most of the nanocapsules prepared were in the particle size range 500–1500 nm diameter. They were able to hold 55–74% of the marker initially present in aqueous solution. In-vitro dissolution studies showed that release of marker was retarded variably in an increasing order from nanocapsules containing MCA, ECA then BCA. Increasing the concentration of the monomer in the nanocapsules led to retardation of marker release.

Recent colloidal delivery systems such as liposomes, nanoparticles and nanocapsules show potential promise as a means of delivering a drug to its site of action efficiently, thereby minimizing any unwanted toxic effects. The production of nanocapsules and nanoparticles by solution polymerization (Couvreur et al 1979a, b), or micellar polymerization (Birrenbach & Speiser 1976; Couvreur et al 1977) has been reported in addition to cross-linking polymerization of coacervated natural macromolecules (Widder et al 1979; Oppenheim & Stewarts 1979). Trials have been carried out to prepare polalkyl cyanoacrylate nanoparticles (Couvreur et al 1979a, b; Kante et al 1980). In addition the use of polyalkyl cyanoacrylate for the microencapsulation of aqueous solutions of proteins has been investigated (Florence et al 1976, 1979; Wood et al 1981). We now discuss the possibility of using these polymers for the preparation of nanocapsules by interfacial polymerization.

Materials and methods

Doxorubicin HCl (Sigma Chemical Co., St Louis, USA), fluorescein (Aldrich Chemicals), methyl cyanoacrylate (MCA), ethyl cyanoacrylate (ECA), butyl cyanoacrylate (BCA) (Loctite Co., München, W. Germany), Arlacel A, and polysorbate 20, were used as received.

Preparation of polyalkyl cyanoacrylate nanocapsules

The marker substance doxorubicin or fluorescein (10 mg) was dissolved in 2 ml of distilled water, the solution was mixed with 20 ml of 5% Arlacel A in a cyclohexane–chloroform mixture (4:1), and the system was sonicated at 40 W for 30 min with continuous cooling on ice. The calculated amount of alkyl cyanoacrylate dissolved in 10 ml of the same organic solvent mixture was added to the emulsified system, and stirred with a magnetic stirrer at 1200 rev min^{-1} for 3 min. An aliquot of the organic solvent mixture (40 ml) was added to quench the reaction and to reduce the possibility of polymerization occurring between the formed nanocapsules. The system was then centrifuged at 14 000 rev min^{-1} for 10 min to remove supernatant. The sedimented nanocapsules were washed with 20 ml of aqueous 5% polysorbate 20 in 50% aqueous ethanol by mixing with a homogenizing head, recentrifuged, rewashed with 20 ml of aqueous 5% polysorbate 20 solution, then recentrifuged. The final colloidal dispersion was prepared by homogenizing the sediment in 20 ml of 1% aqueous polysorbate 20 solution using the homogenizing head for 5 min, leaving overnight, then filtering through a sintered glass filter G3.

Investigation of the nanocapsules

Marker entrapment by the nanocapsules. Nanocapsule dispersion (10 ml) was titrated with 2 M sodium hydroxide solution to pH 9·0 to dissolve the polymer coat and give a completely clear solution which was then measured for its drug content spectrophotometrically for fluorescein, and spectrofluorophotometrically (Zeiss) for doxorubicin. The determinations were carried out against a blank prepared in the same manner using nanocapsules without marker.

Particle size determination of the nanocapsules. The nanocapsule dispersion containing marker was sonicated for 2 min, then sprayed over glass slides. The samples were vacuum dried, coated with a carbon-gold layer (about 50 nm thick), then again vacuum dried before scanning with a scanning electron microscope. The diameters of the nanocapsules were determined from the photomicrographs, at least 150 entities from three different mounts being counted.

In-vitro release of marker from the nanocapsules. Both the free marker permeability and its release from the nanocapsules were measured by the cellulose membrane method. Samples of the nanocapsule dispersions (4 ml) were transferred to a glass tube with a cellulose membrane closing its lower end (3 cm internal

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diameter). The tube with its contents was dipped in 20 ml phosphate buffer of pH 7·2 in a glass beaker and incubated at 37 °C in a thermostatted water bath. The pH of the buffer system was continuously adjusted to 7·2 ± 0·5 with 2 M sodium hydroxide solution, and it was continuously stirred at 50 rev min^{-1}. Samples (0·5 ml) of the solution were withdrawn at various times for analysis and each was replaced by an equal volume of phosphate buffer solution.

Results and discussion

Most of the previous work on polyalkyl cyanoacrylates has been concerned with nanoparticles, and this has shown that the drug is taken up by surface adsorption. The preparation of polyalkyl cyanoacrylate microcapsules was described by Wood et al (1981). In the present work, trials to obtain polyalkyl cyanoacrylate nanocapsules met with difficulties arising from the choice of solvent system for the different alkyl cyanoacrylates; but with careful selection of the system for the different alkyl cyanoacrylates, nanocapsule dispersions were obtained as colloidal suspensions which were stable, without visible precipitation, for more than one month. These products showed homogeneous populations of nanocapsules which appeared as almost perfect spheres (Fig. 1), having the dimensions stated in Table 1. It is clear that increasing the amount of the monomer led to an increase in coat thickness. By calculation, it can be concluded that the emulsification process gave w/o emulsions of globule diameter around 0·7 μm. On the other hand, the coat thickness produced in the presence of the different polyalkyl cyanoacrylates showed slight differences, increasing in the order polyMCA, polyECA, polyBCA. These differences follow the same sequence with either marker.

Entrapment of marker by the nanocapsules

A gradual increase in fluorescein entrapment was noticed with increasing coat thickness when polyMCA was used (Table 1). It was found that use of monomer weights of half, equal and double the amount of that of the aqueous phase led to entrapment of marker with the ratios 1·00 : 1·73 : 3·03. This increased entrapment may be due to resistance of thicker coats to the washing processes. Methyl-, ethyl-, and butyl-cyanocrylate led to increased entrapment according to the ratios 1·00 : 1·33 : 1·42. In the preparation of doxorubicin nanocapsules, the same sequence of alkyl cyanoacrylates gave the ratios 1·00 : 1·22 : 1·41. The higher cross-linking of the polymers prepared by the higher homologues of alkyl cyanoacrylates explains the increased entrapment of marker previously illustrated. In comparison with doxorubicin entrapment by polyMCA nanoparticles prepared according to the method of Couvreur et al (1979a), the amount of marker

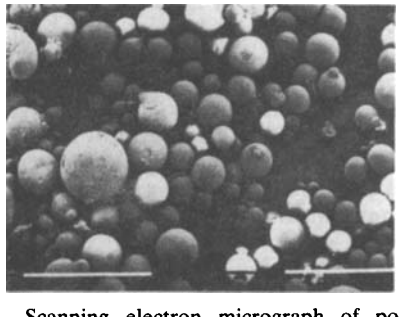

FIG. 1. Scanning electron micrograph of polymethyl cyanoacrylate nanocapsules containing doxorubicin HCl, ×5000.

Table 1. Mean particle diameters, drug entrapment, and drug release rate constants of the different polyalkyl cyanoacrylate nanocapsules.

	Mean particle diameter (μm)	Drug entrapment (%)	Release rate constant ($h^{-1} \times 10^3$)
Fluorescein permeability	—	—	1182·805
Fluorescein in:			
polyMCA nanocap. (33% MCA).	0·801 ± 0·30	28·87	98·197
(50% MCA).	0·882 ± 0 37	50·07	59·119
(67% MCA).	1·050 ± 0·45	87·57	40·291
polyECA nanocap. (50% ECA).	0·887 ± 0·39	66·67	27·938
polyBCA nanocap. (50% BCA).	0·903 ± 0·38	71·37	24·648
Doxorubicin permeability	—	—	115·205
Doxorubicin in:			
polyMCA nanocap. (50% MCA).	0·781 ± 0·28	52·67	16·368
polyECA nanocap. (50% ECA).	0·854 ± 0·29	64·33	9·648
polyBCA nanocap. (50% BCA).	0·931 ± 0·31	74·03	7·525
Doxorubicin in PMCA nanopart.	0·213 ± 0·015	9·71	65·637

From solutions containing 5 mg ml^{-1} of either of the two drugs.

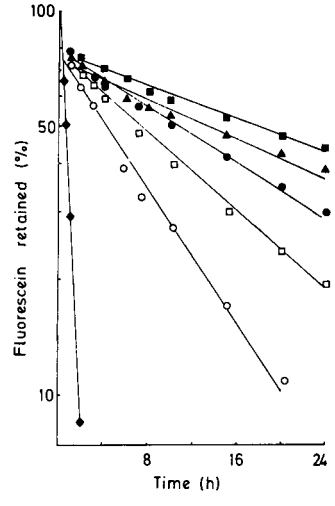

FIG. 2. First-order plot of fluorescein release in phosphate buffer of pH 7·2 from its polyalkyl cyanoacrylate nanocapsules. Key: pure fluorescein permeability (◆), polymethyl cyanoacrylate nanocapsules containing 33·3% (○), 50% (□) and 66·7% (●) of the monomer; polyethyl cyanoacrylate nanocapsules (▲); polybutyl cyanoacrylate nanocapsules (■).

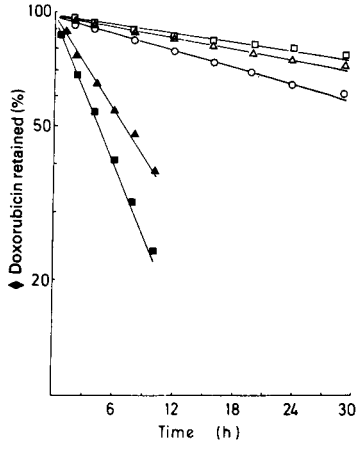

FIG. 3. First-order plot of doxorubicin HCl release in phosphate buffer of pH 7·2 from its polyalkyl cyanoacrylate nanocapsules. Key: pure doxorubicin HCl permeability (■); polymethyl cyanoacrylate nanoparticles (▲); polymethyl cyanoacrylate nanocapsules (○); polyethyl cyanoacrylate nanocapsules (△); and polybutyl cyanoacrylate nanocapsules (□).

entrapped by the polyalkyl cyanoacrylate nanocapsules was 5–8 times greater than that of the polyMCA nanoparticles.

Marker release

Two substances were used to verify the release pattern from the prepared products. Fluorescein was used as a marker to show the effect of coat thickness using MCA, as well as the effect of the different polyalkyl cyanoacrylates at the same coat thickness. Doxorubicin was incorporated into the different polyalkyl cyanoacrylate nanocapsules and its release patterns investigated using for comparison doxorubicin in nanoparticles prepared as described in the literature. The results of release of either fluorescein or doxorubicin (Figs 2, 3) were statistically evaluated by the least squares method. First order kinetic patterns were followed by all the release data obtained (Table 1). With MCA it was found that increasing the monomer concentration decreased the release rate constant. Assuming the same emulsion globule size during nanocapsule preparation, the particle size increase on increasing monomer concentration indicates increased coat thickness. Calculations showed that the release rate constant is directly proportional to the reciprocal of coat thickness. In addition, fluorescein release from nanocapsules prepared in-situ using the homologous series methyl, ethyl and butyl cyanoacrylate, decreased as the alkyl chain length increased. As has been stated, higher alkyl homologues led to highly cross-linked membranes which explains such release behaviour. Doxorubicin nanocapsules followed a similar pattern to that of fluorescein, but the release rate of doxorubicin through the same polycyanoacrylate coat membrane was slower. The permeability of both substances through the cellulose membrane used showed the same behaviour. Molecular volume and mobility of drug molecules in solution, as well as membrane interaction may explain the stated diffusion behaviour.

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